

Research paper

Linking field performance to initial morphological traits of thuja seedlings in a semiarid Mediterranean area

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Abstract. Forest seedling size is still a subject of debate in plant production for reforestation programs in a semiarid Mediterranean area. The aim of the study was to assess the effect of initial morphological traits of *Tetraclinis articulata* (Vahl.) Mast. containerized seedlings on survival rate and growth performance after 11 months of outplanting in a semiarid Mediterranean site. The rooting depth of dead and alive seedlings was also explored to determine the effective depth for survival optimization and consequently to characterize the critical minimal soil water content necessary for seedlings to overcome drought season. The survival probability of *T. articulata* seedlings was strongly influenced by the initial seedling collar diameter, showing an increasing pattern with a critical minimum of 3.24 mm. The sturdiness index affected the survival probability, showing a trend downwards with a critical maximum of 5.93 cm mm⁻¹. The shoot height relative growth rate of the seedlings was significantly affected by the initial morphological class, giving more advantage to small seedlings, while the collar diameter relative growth seemed to be insensitive to the initial seedling size. The evaluation of the rooting depth in relation to seedling survival allowed the characterization of a minimum depth of 57.74 cm that the roots must exceed in order to reach the wettest soil layers; this soil depth corresponds to a minimum critical gravimetric water content of 12% below which the roots cannot survive. These results suggest that in semiarid regions, where rainfall is scarce and soil moisture is uncertain, planting short-stemmed seedlings with a large collar diameter can enhance survival and shoot growth during the first year after planting.

Key words: *Tetraclinis articulata*, seedling quality, seedling survival and growth, drought, rooting depth.

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Introduction

The quality of forest seedlings in general and plant size in particular is a very important issue in plant production for reforestation programs (Rose *et al.*, 1990; Olié *et al.*, 2019). Seedling quality can be defined as the ability of plants to survive and grow after transplanting in a specific environment (Ritchie, 1984; Ritchie *et al.*, 2010; Wilson & Jacobs, 2006). In this context, an important challenge for nursery manager is to produce plants with attributes or traits that offer the best chance of survival in the field (Burdett, 1983; Mattsson, 1997; Grossnickle, 2012). Assessing forest seedling quality is critical for successful reforestation and plantation establishment. The timing of

assessment depends on the stage of seedling development and the purpose of the evaluation (e.g. nursery management, outplanting readiness, or post-planting survival) (Haase, 2008; Jacobs & Landis, 2009; Grossnickle & MacDonald, 2018a). To establish effective quality standards for a specific species, two factors need to be considered: first, quality attributes of the plants should be selected based on the pedo-climatic conditions of the target planting sites (arid or humid zone, soil fertility, competition from existing vegetation, etc.) (Pinto *et al.*, 2011); second, the choice of standards for the field should be confirmed over a long period (Vallejo *et al.*, 2012). There is no single, universal test for forest seedling quality assessment, but separate tests for each species

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should be developed for different regions and with different cultivation techniques (Grossnickle & Folk, 1993). The morphological attributes of seedlings, such as shoot height (cm), root collar diameter (mm), sturdiness (height to collar diameter ratio) and root/shoot ratio (g/g) are widely used to assess seedling quality at lifting time in nurseries (Grossnickle, 2012; El Haddadi *et al.*, 2022). They are easy to measure and generally exhibit good correlation with field performance (Bayley & Kietzka, 1997; Jacobs *et al.*, 2005). Measurements of root system morphology are also consistent in determining seedling quality and field performance (Davis & Jacobs, 2005; Cortina *et al.*, 2008). However, these measurements are destructive, laborious and time-consuming (Grossnickle & MacDonald, 2018b).

In the Mediterranean environment, water stress is the main limiting factor for the survival of plantations, and therefore for the success of forest restoration projects (Moles & Westoby, 2004). Field survival and growth of many planted species are frequently very low (Vilagrosa *et al.*, 2003; Navarro *et al.*, 2006; Vallejo *et al.*, 2006). This reality has encouraged foresters for decades to prefer the use of short-stem seedlings with a high root/shoot ratio, which they consider adapt better to arid conditions than large plants whose root/shoot is low (Royo *et al.*, 1997; Trubat *et al.*, 2011). Large seedlings require more water than those with opposite traits (Leiva & Fernández-Alés, 1998). Under dry conditions, planting small seedlings is considered as a conservative strategy that can reduce transplanting shock by minimizing shoot water loss (Lamhamedi *et al.*, 1997; Trubat *et al.*, 2004; Trubat *et al.*, 2011; Cortina *et al.*, 2013; Ovalle *et al.*, 2016). On the other hand, other authors have observed that large plants have recorded a better success rate and improved growth in the field than small plants (Oliet Palá *et al.*, 1997; Puértolas *et al.*, 2003; Luis Díaz *et al.*, 2004; Villar-Salvador *et al.*, 2004; Oliet *et al.*, 2005; Tsakaldimi *et al.*, 2005). In most cases, no strong relationship has been confirmed between post-planting success or growth rate and plant size (Grossnickle & MacDonald, 2018b). In the same vein, Trubat *et al.* (2010) has found that nutrient management in the nursery showed a strong potential for modifying morpho-functional characteristics of *Quercus suber* L. seedlings but not field performance. These contradictory results emerge from excessive dependence of seedling performance on environmental conditions (Grossnickle, 2012). The effect of environmental factors under field experiments in dry areas indeed override the effect of plant size (Cortina

et al., 2013). However, it was concluded that the standards in use for regulating the shoot height of certain Mediterranean forest plants must be revised downwards according to Navarro *et al.* (2006). Nevertheless, the question remains: what is the necessary shoot height and collar diameter of target seedlings for a particular species in a particular site?

Seedling establishment at planting sites is a critical stage in the life cycle of plants as the highest mortality rates are often associated with this phase (Fenner, 1987). Post-transplant establishment is severely hampered by long dry summer periods (Herrera, 1992). Field establishment may be improved through nursery treatments that ensure high quality seedlings with a deep and well-structured root system (Chirino *et al.*, 2008; Tsakaldimi *et al.*, 2009). A deep root system can improve water uptake and increase the probability of survival in Mediterranean areas (Canadell & Zedler, 1995; Lloret *et al.*, 1999; Bocio *et al.*, 2004) because of access to water from deep soil horizons where some water is available even in the driest seasons, avoiding the summer drought stress and the indirect water stress due to plant competition (Pinto *et al.*, 2012). A positive correlation has been observed between soil depth, soil moisture and seedling survival (Padilla & Pugnaire, 2007). Therefore, facilitating deep plant rooting may be more important than increasing the volume of tilled soil (Grantz *et al.*, 1998; Padilla & Pugnaire, 2007). In this context, the search for the optimal depth of planting holes in the field should also be considered in conjunction with the root plug length of containerized seedlings in the nursery.

Forest seedlings are vital for reforestation and sustainable forest management, particularly in regions like Morocco, which has a diverse range of forest ecosystems, including argan, cedar, pine forests, and oak woodlands. Although quality standards for forest seedlings are a critical management tool for ensuring survival and growth after planting, research on defining these standards remains scarce and deals with general aspects (Abourouh, 1994; Lamhamedi & Fortin, 1994) or focuses on specific issues (Ferradous & Hafidi, 2018; Zine El Abidine *et al.*, 2016a; Zine El Abidine *et al.*, 2016b). The lack of standards may imply reliance on international or regional practices, such as those from southern Europe (Spanish standards) (Bouderrah *et al.*, 2017). However, this has no practical implications for nursery managers or forest planters.

Tetraclinis articulata, known as thuja or Berber cedar, is a Mediterranean re-sprouting

Cupressaceae which extends from north-western Africa to south-western Europe, covering more than one million hectares (Quézel, 1980; Charco, 1999; Sánchez-Gómez *et al.*, 2013), mainly located in Morocco with 671,100 ha (FAO, 2015). Thuja forests are sources of highly socio-economic and ecological valuable services in semiarid environments but their natural regeneration is facing multiple constraints related to overgrazing, drought and soil degradation (Esteve-Selma *et al.*, 2010). The current governmental management approach considers planting high-quality containerized seedlings as an alternative in order to assist and accelerate the natural regeneration process. In this context, there is a need to develop target values for the morphological traits of thuja seedlings in nurseries to optimize outplanting survival and growth.

We hypothesize that the morphological characteristics of thuja seedlings at the nursery lifting stage could impact their drought tolerance, thus influencing their field establishment. The objectives of this study were:

- First, to characterize the influence of the morphological traits of thuja initial seedlings (shoot height, collar diameter and sturdiness index) on field survival and growth after 11 months post-transplanting in a semiarid Mediterranean area;
- Second, to determine the minimal critical soil water content that optimizes survival rate;
- Third, to highlight the relationship between initial morphological traits and the development of deep root systems.

Material and Methods

Plant material and seedling production in the nursery

T. articulata seeds were collected from the Ouchket forest (33.782397°N, 05.879016°W; 265 m elevation, P05 stand) on September 25, 2018 and stored in a climate chamber at 4°C. Seedlings were raised at Sidi Amira Forest Nursery, Salé, Morocco (34.050341° N, 06.671741° W; 130 m elevation) under outdoor conditions with shading nets and auto-spray irrigation with a mean annual rainfall of 534 mm and mean temperatures: $T_{\max} = 27.3^{\circ}\text{C}$ and $T_{\min} = 8.2^{\circ}\text{C}$ (Figures 1(i) and 1(ii)). To achieve this, seeds were pre-germinated and sown in early March, 2019 in rigid plastic containers (50 cells of 400 ml, 5 x 5 cm section and 16 cm cavity depth with anti-spiraling system) filled up with a mixture (4:1) of peat and local material substrates (sandy topsoil). The peat (TS3 Klamann brand) was fertilized in the factory with NPK: 14–10–18 (1.5 kg fertilizer m^{-3} of peat). The watering regime was moderated according to the seedlings' water demand. Using a digital balance, irrigation was determined by gravimetric water content (Lamhamedi *et al.*, 2006) and maintained equally across all containers at 85% during establishment (mid-March to mid-May), 75% during rapid growth (mid-May to August 31th) and 65% during hardening (September 1st to lifting time). During the whole growing season (from March 16th to December 11th, 2019), normal management practices were carried out with a fortnightly fertilization with the 20–20–20 NPK fertilizer. Before leaving the nursery, thuja seedlings received a saturating irrigation.

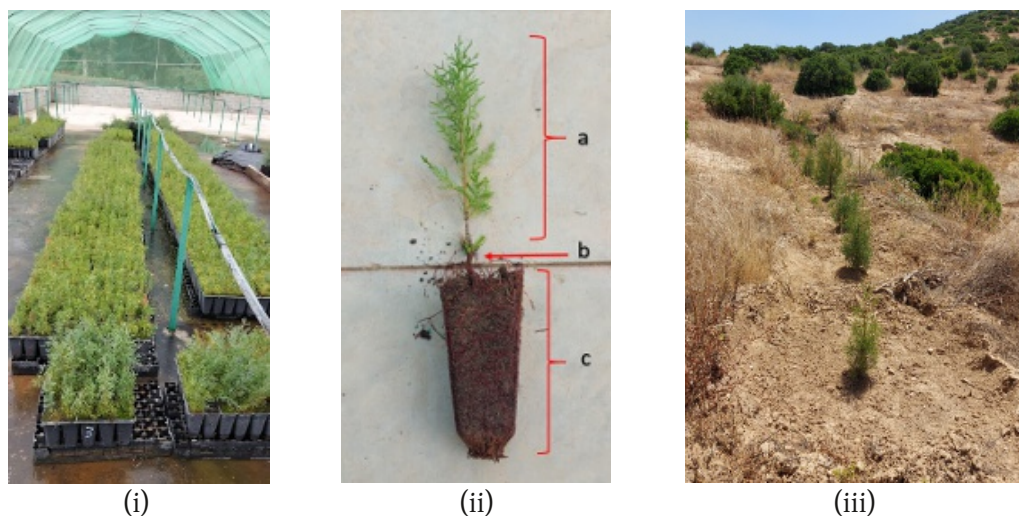


Figure 1. Thuja seedlings from the nursery to the field: (i) at lifting time in the nursery (December 10, 2019), (ii) after extraction from the container showing: [a] shoots, [b] collar, [c] root plug, (iii) after outplanting in the field (July 15, 2020).

Field site conditions and experimental design

Site description: The experimental protocol was set up in the same forest where the thuja seeds had been collected – the P05 stand of Ouchket Forest where a 5.5 ha parcel has been fenced to prevent herbivores and reserved for the needs of the present experiment. It is a Berber cedar matorral on carbonated shallow soils derived from marl, with a slope ranging between 20% and 30%, exposed southwest. The climate is classified as Mediterranean, exhibiting temperate conditions and semiarid characteristics (Oued Beht weather station, 10 km northwest of the site) with a mean annual rainfall of 471 mm, $T_{\max} = 35.5^{\circ}\text{C}$ and $T_{\min} = 6.9^{\circ}\text{C}$. The site has a low vegetation cover (10% to 15%) made up of an understory of *Pistacia lentiscus* L., *Ziziphus lotus* (L.) Lam., *Olea europaea* var. *oleaster* (Hoffmanns. & Link) A.DC., *Rhus pentaphylla* (Jacq.) Desf. and *Chamaerops humilis* L., interspersed with a few old *Tetraclinis articulata* and *Pistacia atlantica* Desf. trees. The regressive evolution of the site vegetation has led to the proliferation of herbaceous species such as *Urginea maritima* (L.) Baker, and *Asphodelus macrocarpus* Parl.

Climatic variables: The rainfall received at the experimental site during the entire planting season (October 1st, 2019 to September 30th, 2020) totaled 393.4 mm, which represents a 16.4% reduction compared to the annual average. The planting window was decided when the cumulative rainfall received at the site exceeded 50 mm (December 12th, 2019) (Figure 2).

Early planting gave outplanted seedlings a better chance by taking advantage of more than 80% of the rain during the planting season. During the dry season (mid-May to mid-October 2020), cumulative rainfall did not exceed 49 mm, distributed in the form of ephemeral thunderstorms the daily amount of which hardly exceeded 1 mm (Figure 3). Maximum temperatures peaked at 43°C twice in July and a third time in August. In summary, the seedlings experienced a long and extremely dry summer season.

Experimental design: The land was terraced following the contour lines of a bulldozer into 1.5 to 2 m wide benches (swales) and spaced approximately 5 m apart. The bench body was made into a trench of 0.5 m in depth to allow rainwater harvesting (Figure 4). Planting holes of 0.4 x 0.4 x 0.4 m were manually dug on the ridge of the benches in order to facilitate root development through the loose soil. Adjacent planting holes on the same bench were spaced 2 m apart (Figure 1(iii)). Taking into account the heterogeneity of the environment, and to have a better representativeness of the sampling protocol, a completely randomized design (CRD) was adopted. 5,202 seedlings were outplanted from 12th to 15th December 2019. Outplanted seedlings were divided into 153 plots where each one was made up of 34 adjacent seedlings along the contour line. 51 plots were randomly sampled to form the observed population where each seedling was identified with a label. Finally, the total number of sampled seedlings was 1,734.

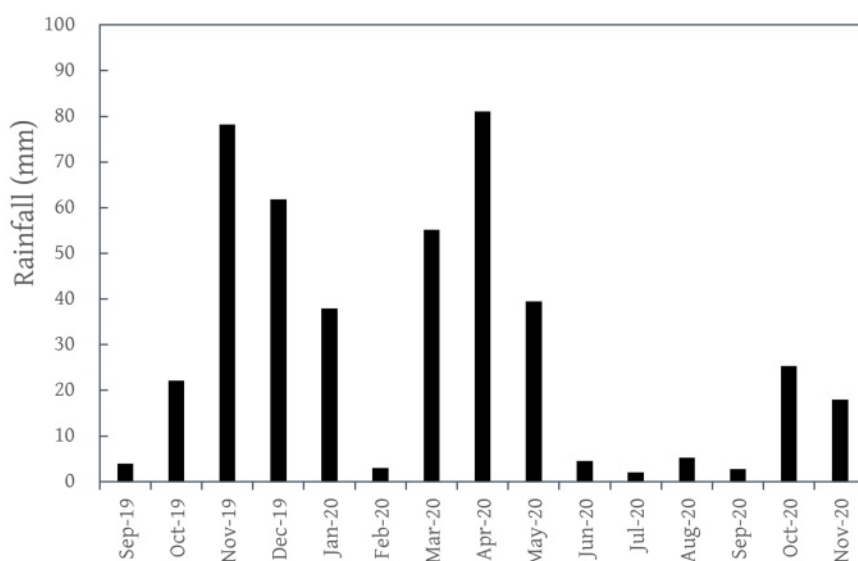


Figure 2. Average monthly rainfall (mm) during the entire planting season (October 1st, 2019 to October 30th, 2020) collected in the meteorological station of Oued Beht, Khémisset. Note how the plantation window (December 12th, 2019) was decided after the occurrence of more than 100 mm cumulative rainfall.

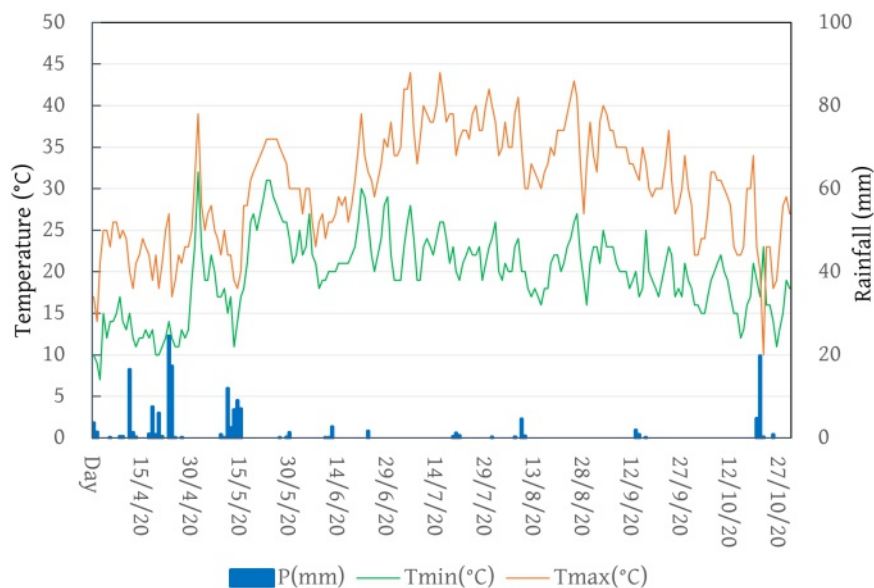


Figure 3. Variation of daily rainfall (mm) and temperatures ($T_{\max}$ and $T_{\min}$ (°C)) from April 1st to October 30th, 2020. It is noteworthy that the dry period (from mid-May to mid-October 2020) coincided with the hottest season; data collected in the meteorological station of Oued Beht, Khemisset.

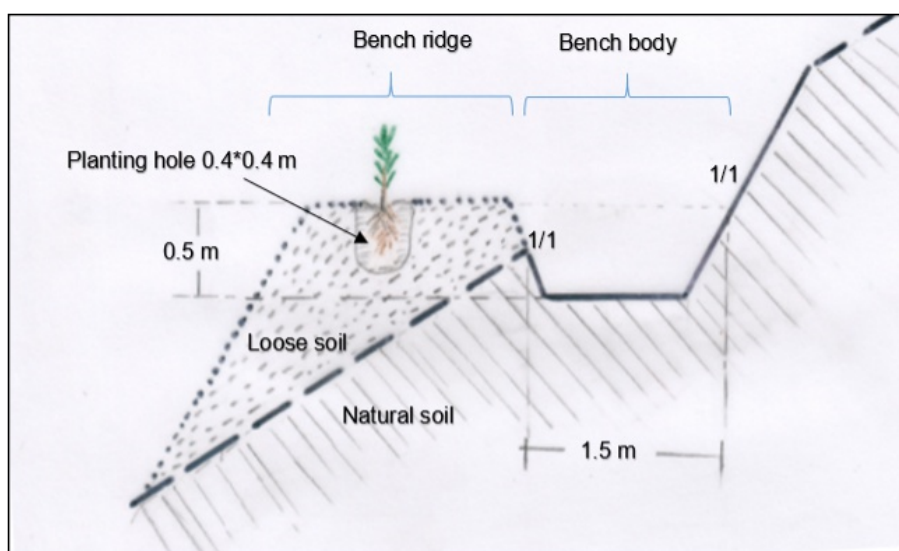


Figure 4. Profile of a forest bench showing how seedlings were planted on the bench ridges.

Measurements of the morphological traits of outplanted seedlings and field survival

One week after outplanting, the initial morphological traits of observed seedlings were assessed:

- shoot height H_0 (cm): vertical distance from ground level to terminal leader tip using a 0.1 cm-accuracy ruler;
- basal diameter D_0 (mm), hereafter called “collar diameter”: measured at 0.5 cm above the ground line using an electronic caliper with 0.1 mm accuracy;
- sturdiness index $SI_0 = H_0/D_0$ (cm mm⁻¹).

Measurements of the initial morphological traits of the seedlings were performed in the

field just after outplanting to reduce the “planter effect” and re-measurement errors.

11 months post-planting (9th to 12th November 2020), the morphological traits of the same seedlings were re-measured with reference to their identification label designated hereafter (H_1 , D_1 and SI_1).

The relative growth rate (RGR) for shoot height and basal diameter were calculated for living seedlings using the following formula (Kramer & Kozłowski, 1979; Hunt *et al.*, 2002):

$$RG(X) = (\ln(X_1) - \ln(X_0)) / \Delta t, \quad (1)$$

where X_0 and X_1 denote morphological traits

measured at the time of the first (outplanting time) and second assessment (11 months after outplanting) of the parameter X , respectively, and Δt is the time interval between the two measurements.

Field survival was assessed monthly and every plant was noted according to the binary system (0; 1) = (dead plant; alive plant). Whenever the vitality of a plant is in doubt, the measurement of its midday water potential was carried out using a pressure chamber (SKPM 1405/80, Skye instruments Ltd). A *Tetraclinis articulata* seedling was considered dead when its midday water potential measured on branches was below -7 MPa (Oliveras *et al.*, 2003). Seedling survival was graphically described to show when the survival rate declined more drastically.

Soil moisture content and plant rooting depth assessment

The relationship between soil moisture content variations and seedling survival was investigated in two steps:

First, soil moisture was measured on five profiles (Bélanger & Van Rees, 2007) randomly dug on benches' ridges on the 15th of each month throughout the drought period (mid-May to mid-October). In each profile, four soil samples were taken from each depth horizon: 10, 30, 50, 70 and 90 cm using a forestry auger and aluminum boxes. The soil moisture considered is the gravimetric moisture content Hg (%) (Gardner, 1986):

$$Hg (\%) = (M_{\text{wet}} - M_{\text{dry}}) / M_{\text{dry}} * 100, \quad (2)$$

where M_{wet} is the weight of the fresh soil sample (electronic balance with 0.001 g precision) and M_{dry} is the weight of the soil sample after oven drying at 104°C for 24 hours.

Second, rooting depth (distance between the ground line and the bottom of the taproot after planting) was measured on 62 plants of different sizes randomly selected from those involved in the repetitive measurements of morphological traits. For this purpose, 31 live plants and 31 dead plants (reported dead during the drought season between August 15th and September 15th, 2020) were carefully extracted from the soil, and the taproot length was measured for each plant on September 15th, 2020.

Data analysis

As a prospective method, the logistic regression model with a 95% confidence interval was used to assess the effect of the initial morphological traits of the seedlings (explanatory variable) on

their survival (binary response variable). The logistic function is the following:

$$P_{(x)} = 1 / (1 + \exp(-(\beta_0 + \beta_1 x))), \quad (3)$$

where P is the probability of survival, x is the measured variable, β_0 and β_1 are model parameters, $\mu = -\beta_0/\beta_1$ is a location parameter represented by the midpoint of the curve, where $P(\mu) = 0.5$ (Hosmer & Lemeshow, 2000; Peng & So, 2002). The location parameter is of particular interest in the logistic regression as it represents the "target" value of the explanatory variable that accounts for 50% of the survival rate. The odds ratio ($OR = \exp(\beta_1)$) predicts how the probabilities of survival vary with increases in the explanatory variable (Jaccard, 2001).

The K-means clustering technique was used to group the data on similar initial morphological traits of the seedlings into clusters so that the similarities among data members within the same cluster are maximal while the similarities among data members from different clusters are minimal (Forgy, 1965; MacQueen, 1967; Kaufman & Rousseeuw, 2009). This technique allowed assigning each seedling to an initial morphological class (cluster) i characterized by a centroid (H_{0i} ; D_{0i} ; SI_{0i}) using the minimal Wilks lambda classification criterion and minimal intra-cluster variance. Seedling survival within initial morphological clusters after 11 months of outplanting was registered for each observed plot. Seedling survival and the relative growth rate in relation to initial morphological traits were compared by means of analysis of variance (one-way ANOVA).

The relationship between rooting depth and survival was assessed by the logistic regression model while the relationship between rooting depth and initial morphological traits was characterized by linear regression analyses using the determination coefficient (R^2).

All of the statistics were performed using the XLSTAT software (v2016 02.27444), and the results are reported as mean $\pm$ standard deviation. The distribution was tested for normality by the Shapiro-Wilk test ($p \leq 0.05$), and the homogeneity of variances was tested by Levene's test. When normality was not acquired, data were analyzed according to the non-parametric Kruskal-Wallis test at 5% probability and therefore Dunn's Multiple Comparison Test was used to separate the means of variables.

Results

Initial morphological traits of the out-planted seedlings

Morphological quality assessment at out-planting time showed that the seedlings were characterized by a wide range and heterogeneity of morphological traits (Table 1): Shoot height varied between 4 cm and 53 cm with an average of 20.58 cm and the coefficient of variation (CV) exceeded 40%. Similarly, the collar diameter varied between 1 mm and 9 mm with an average of 3.37 mm and 23.14% CV. The sturdiness index, which reflects the stocky or spindly nature of the seedlings, varied between 2.26 cm mm⁻¹ and 10.7 cm mm⁻¹ with a CV of 25%. The relationship between different initial morphological traits of the observed seedlings

was illustrated with a linear regression in Table 2.

Shoot height strongly correlated with both collar diameter ($R^2 = 0.72$) and sturdiness index ($R^2 = 0.76$) while the latter weakly correlated with collar diameter ($R^2 = 0.26$).

Seedling clustering based on morphological attributes using the K-means method with four homogeneous clusters, hereafter called classes, is illustrated in Table 3 where each morphological class is characterized by a centroid (H_0 ; D_0 ; SI_0).

The sturdiness index increased with seedling size and the intra-class variance was lower or very close to the global variance which is 6.73, except for the extra-large class which showed great heterogeneity expressed by a variance of 12.47.

Table 1. General morphological traits of thuja seedlings at lifting time in the nursery ($n = 1734$).

Attributes	Min	Max	Mean
Shoot height (cm)	4.01	53.01	20.58 ± 8.25
Collar diameter (mm)	1.07	9.11	3.37 ± 0.78
Sturdiness index	2.26	10.69	5.92 ± 1.48

Table 2. Correlations between different initial morphological traits of thuja seedlings.

Regression model	n	p -value	R^2
$H_0 = -9.507 + 8.896 \cdot D_0$	1734	$p < 0.0001$	0.72
$H_0 = -8.323 + 4.875 \cdot SI_0$	1734	$p < 0.0001$	0.76
$SI_0 = 2.608 + 0.978 \cdot D_0$	1734	$p < 0.0001$	0.26

Table 3. Initial morphological classes of thuja seedlings at outplanting time using the K-means method. IMC: Initial morphological class.

IMC	Seedling size	Centroid			Class weight	Intra-class variance
		H_0 (cm)	D_0 (mm)	SI_0 (cm mm ⁻¹)		
A	Small	10.8	2.52	4.35	513	6.333
B	Medium	18.25	3.31	5.57	460	5.100
C	Large	24.93	3.76	6.7	435	4.641
D	Extra-large	32.75	4.26	7.74	326	12.477
Total					1734	

Seedling survival

Despite the low amount of rainfall received at the experimental site during the 2020 planting campaign, the good distribution of rainfall, particularly winter and spring rains, facilitated seedling establishment and improved the overall survival rate, which reached 82%. The outplanted seedlings were able to overcome the transplantation shock, as mortalities did not start to appear until early August 2020 under drought conditions (Figure 5). Seedling survival within morphological classes showed a significant effect with $p < 0.0001$ (Figure 6). Small and extra-large seedlings resulted in similar survival rates of 73.4% and 73.2%,

respectively, while medium and large seedlings showed the highest survival rates of 89.8% and 91.06%, respectively.

Initial morphological traits and survival prediction

The initial shoot height, collar diameter and sturdiness index were investigated as simple predictor variables for the first-year survival. In view of collinearity between these three variables, logistic regression was carried out separately for each variable (Table 4). The logistic regression model showed no significant effect of the initial shoot height on field survival ($p = 0.124$) (Table 4). In contrast, the

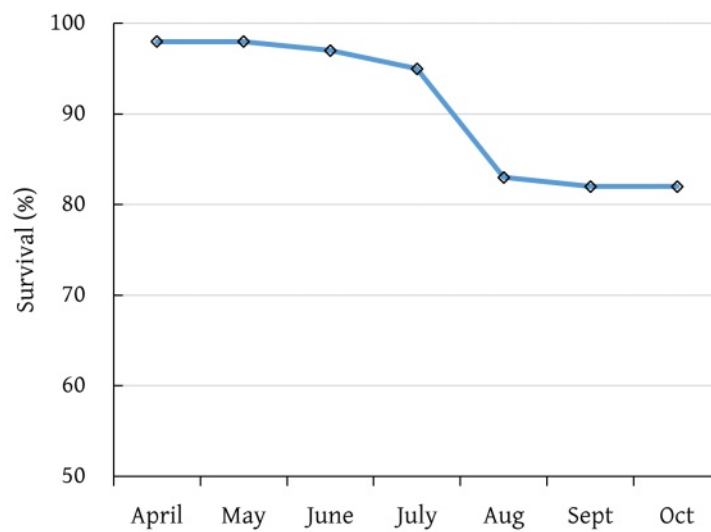


Figure 5. Survival rates of *Tetraclinis articulata* seedlings during the dry period of 2020 showing a remarkable drop between July 15 and August 15, 2020.

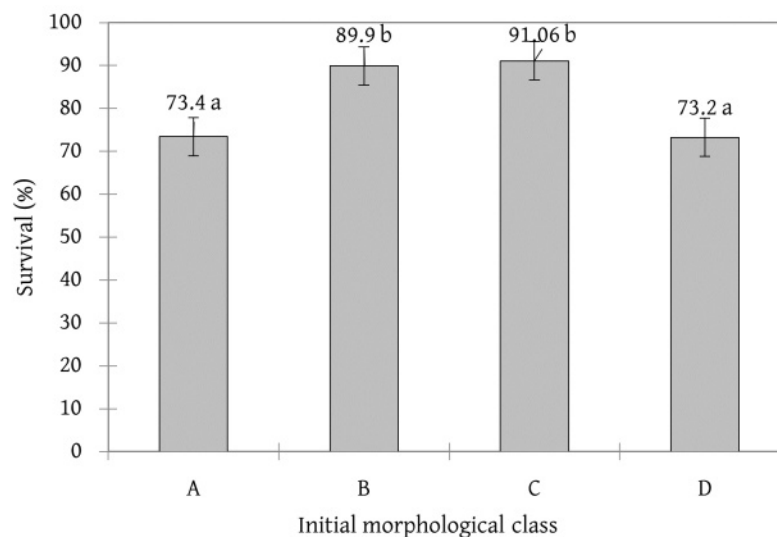


Figure 6. The survival rate of thuja seedlings within initial morphological classes among the 51 observed plots (A: Small, B: Medium, C: Large, D: Extra-large seedlings). Different letters among treatments denote significant differences ($p < 0.05$) according to Fisher LSD test ($n = 204$).

Table 4. Logistic regression model parameters of the survival and initial morphological traits of thuja seedlings: shoot height H_0 (cm), collar diameter D_0 (mm) and sturdiness index SI_0 (cm mm^{-1}) ($n = 1734$). SE: standard error; OR: odds ratio; CI: confidence interval.

Source	β	μ	SE	Wald's χ^2	Pr > χ^2	OR	95% CI for OR	
							Lower	Upper
Constant	-0.158		0.113	1.946	0.163			
H_0 (cm)	0.008		0.005	2.371	0.124	1.008	0.998	1.018
Constant	-1.693		0.197	73.789	< 0.0001			
D_0 (mm)	0.522	3.24	0.059	78.966	< 0.0001	1.685	1.502	1.891
Constant	0.475		0.179	6.991	0.008			
SI_0 (cm mm^{-1})	-0.080	5.93	0.029	7.528	0.006	0.924	0.873	0.978

initial collar diameter positively affected field survival probability with a highly significant odds ratio (OR = 1.685 with $p < 0.0001$) (Table 4). Otherwise, the target collar diameter, which corresponds to 50% of the survival probability was $\mu = D_{0\text{-target}} = 3.24$ mm. The initial sturdiness index registered also a significant effect ($p = 0.006$) on field survival probability but with a decreasing tendency ($\beta_1 < 0$). The odds ratio was OR = 0.924 (Table 4) and the target sturdiness index was $SI_{0\text{-target}} = 5.93$ cm mm⁻¹. The inferential statistical tests for overall models (likelihood ratio, score and Wald tests) yielded a similar conclusion for the given data set with $p < 0.05$. The overall correct prediction of 58.05% for D_0 and 52.11% for SI_0 showed an improvement over the chance level which is 50%. It is worth arguing that collar diameter may be considered a good survival predictor variable and override the effect of other morphological parameters.

Seedling growth assessment

Analysis of variance was used to characterize the effect of the initial morphological character-

istics on the relative growth rate (RGR) of the seedlings. This analysis was undertaken on balanced lots of 200 plants randomly sampled from each morphological class. Since the homogeneity of variances of the RGR(H) and RGR(D) samples were not met using Leven's test ($p < 0.001$ and $p = 0.029$, respectively), a non-parametric Kruskal-Wallis test was undertaken to characterize the effect of each initial morphological class on the relative growth rate of the seedlings. The results in Table 5 show that the initial morphological traits had a highly significant effect ($p < 0.001$) on the seedling shoot height RGR, thus, short seedlings (class A) performed better than taller ones (class B, C and D). In contrast, collar diameter RGR was insensitive to the initial seedling morphological class ($p = 0.263$). Sturdiness index variation showed a significant increase among small seedlings belonging to class A (Table 5) and a significant decrease among large and extra-large seedlings (class C and D), while the seedlings of class B (medium size) did not show significant changes for this parameter ($SI_1/SI_0 \approx 1$).

Table 5. Comparison of shoot height and collar diameter relative growth rates and sturdiness index variation within the initial morphological classes of thuja seedlings using the Kruskal-Wallis test with a 5% significance level. The means are separated by the Dunn procedure. Values followed by different letters within the same column are significantly different. IMC: Initial morphological class.

IMC	Obs.	RGR (H) (cm/cm/month)	RGR (D) (mm/mm/month)	Sturdiness index variation		
				SI_0	SI_1	SI_1/SI_0
A	200	0.033 ± 0.017 c	0.029 ± 0.017 a	4.250 a	4.449 a	1.061 ± 0.171 c
B	200	0.028 ± 0.018 b	0.028 ± 0.018 a	5.472 b	5.442 b	1 ± 0.147 b
C	200	0.023 ± 0.013 ab	0.030 ± 0.014 a	6.696 c	6.649 c	0.938 ± 0.127 a
D	200	0.02 ± 0.014 a	0.031 ± 0.017 a	7.393 d	6.215 d	0.911 ± 0.118 a

Soil moisture, rooting depth and survival

Soil water content during the dry period (Figure 7) reflected the precipitation pattern, with high percentages (> 25%) during late spring that progressively decreased to values below 10% for the first 30 cm of soil depth. Soil water content stayed relatively > 23% in deep soil layers (> 80 cm). The deepest soil layers maintained a relatively high and sustained moisture content during the entire dry season.

The analysis of variance of soil moisture content Hg (%) as a function of soil depth (Sd) at the end of the drought season (Figure 8) resulted in four homogeneous moisture floors (0–20 cm; 20–40 cm, 40–80 cm, and > 80 cm) with the respective soil moisture content of 7.8%, 9.8%, 12%, and 21.7%. A polynomial regression was established between these two

parameters as follows:

$$Hg (\%) = 0.0025*(Sd)^2 - 0.098*(Sd) + 9.2879 \quad (4)$$

with $R^2 = 0.906$ and $n = 100$.

Furthermore, a strong correlation was found between seedling survival and seedling rooting depth (Rd), expressed as a logistic model (Table 6). The probability of survival increases significantly ($p < 0.0001$) with rooting depth, characterizing a target rooting depth: $\mu = Rd_{\text{target}} = 57.74$ cm and odds ratio = 1.1.

Whenever a plant strives to develop its taproot beyond this depth by 1 cm, its chance of survival will be improved by 10%. By exploiting the relationship between soil depth and its moisture content (equation 4), it

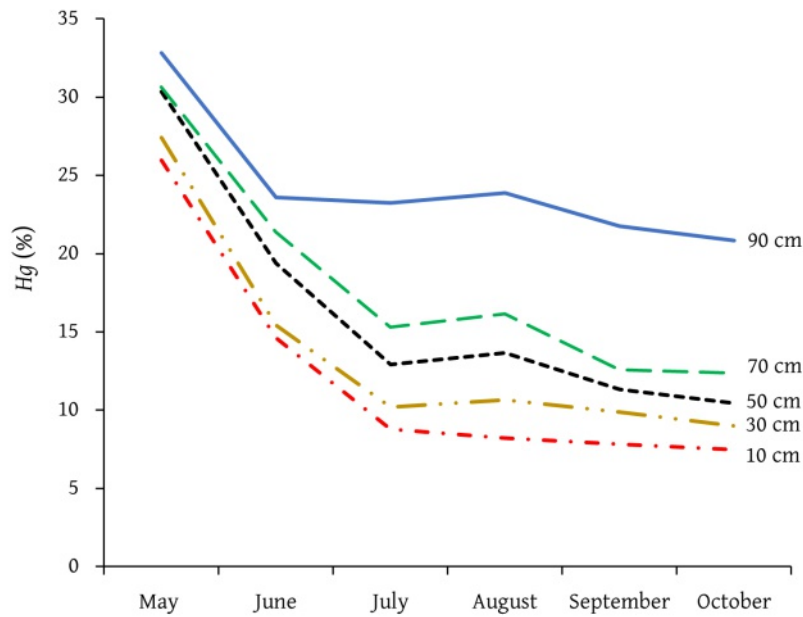


Figure 7. Gravimetric soil moisture content H_g (%) pattern within soil depth (10, 30, 50, 70 and 90 cm) during drought season (May to October 2020).

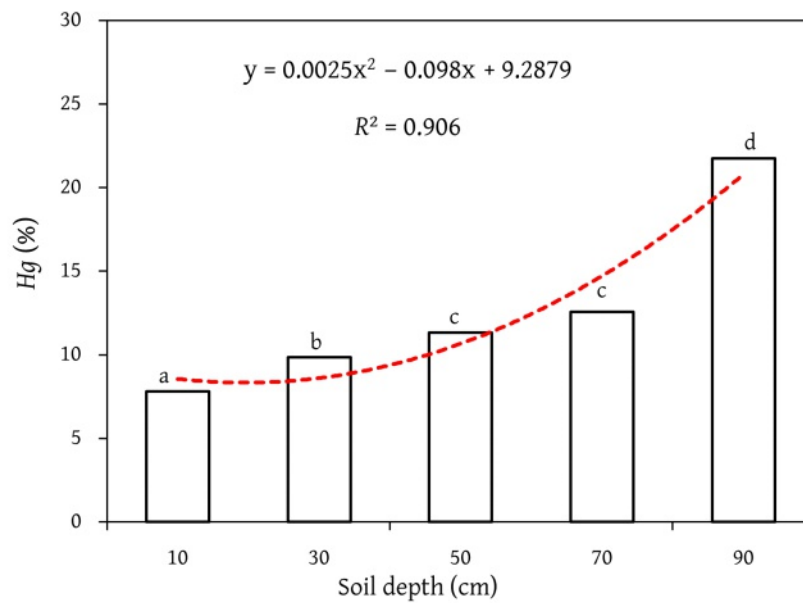


Figure 8. Soil moisture content H_g (%) variation as affected by soil depth (cm) at the end of the drought season (September 15, 2020). Different letters among treatments denote significant differences ($p < 0.05$) according to Fisher LSD test ($n = 100$).

Table 6. Logistic regression model parameters of the survival and rooting depth (cm) ($n = 62$) of thuja seedlings. SE: standard error; OR: odds ratio; CI: confidence interval; Rd: rooting depth (cm).

Source	β	μ	SE	Wald's χ^2	Pr > χ^2	OR	95% CI for OR	
							Lower	Upper
Constant	-5.428		1.542	12.394	<0.0001			
Rd (cm)	0.094	57.74	0.026	13.284	<0.0001	1.1	1.045	1.156

becomes possible to directly associate the probability of plant survival with the soil water content of the deepest soil layer reached by the seedling taproot. Thus, the target soil gravimetric moisture content for thuja seedlings correspond to $11.92\% \approx 12\%$.

Initial morphological traits and rooting depth of seedlings

The single trait models developed to determine

the usefulness of the initial shoot height, collar diameter or sturdiness index for predicting the seedling rooting depth were significant ($p < 0.05$) for all variables. However, the magnitude of variation explained by these models differed substantially: the initial collar diameter accounted for 65% of the variation in rooting depth, while the initial shoot height and initial sturdiness index explained only 23% and 5%, respectively (Figures 9, 10, and 11).

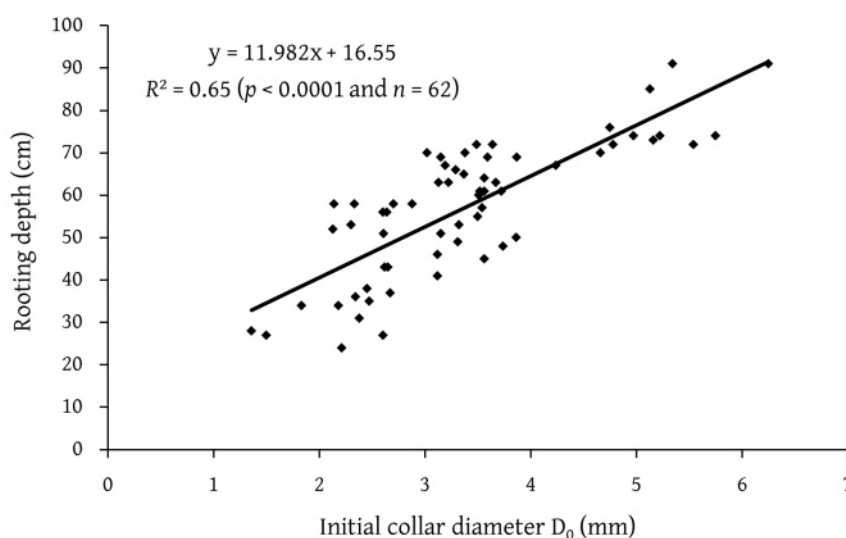


Figure 9. Relationship between initial collar diameter and rooting depth of thuja seedlings after 11 months of outplanting in the field.

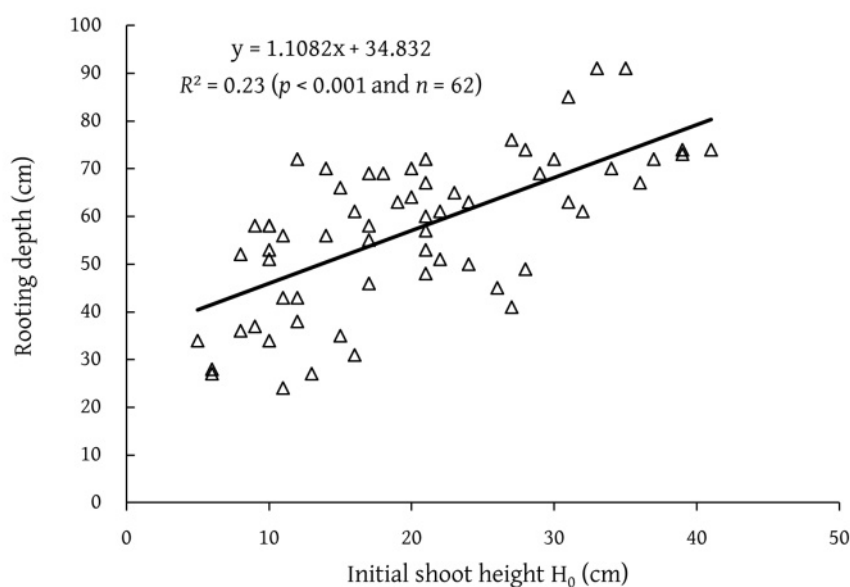


Figure 10. Relationship between initial shoot height and rooting depth of thuja seedlings after 11 months of outplanting in the field.

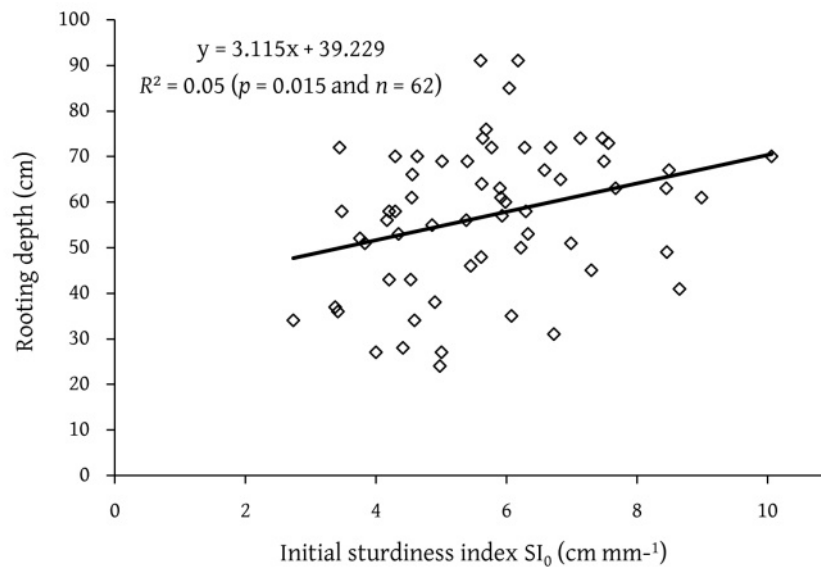


Figure 11. Relationship between initial sturdiness index and rooting depth of thuja seedlings after 11 months of outplanting in the field.

Discussion

The survival probability of thuja seedlings was strongly influenced by the initial collar diameter with a critical minimum of 3.24 mm. Collar diameter has been reported in previous studies as an important predictor of field survival under dry conditions (Zida *et al.*, 2008; Mañas *et al.*, 2009; Tsakalimi *et al.*, 2013). This explains decrease in the survival rate (Figure 6) registered within seedlings belonging to class A (73.4%) the mean collar diameter ($= 2.52 \text{ mm}$) of which was below the critical value of 3.24 mm. Seedling survival was the highest (89% to 91%) for medium and large seedlings (class B and C) for which collar diameter was larger than the critical minimum diameter (Figure 6). On the other hand, plants belonging to the extra-large class (class D) showed a decrease in survival rate (73.2%) in comparison to seedlings of medium and large size (Figure 6), which raises questions. In fact, increasing the collar diameter also increases the height of the stem (Table 3) and thus may allow the expression of the sturdiness index effect. Outplanting survival has been shown to decrease after an optimum root-collar diameter is exceeded (South & Mitchell, 2006) and this is consistent with our results concerning the survival rate drop observed in extra-large seedlings, for which the sturdiness index ($= 7.74 \text{ cm mm}^{-1}$) is much higher than the critical maximum of 5.93 cm mm^{-1} . It is thus desirable to opt for thuja seedlings while observing those two

thresholds. In another research undertaken on a similar Mediterranean coniferous (*Pinus pinea* L.), Dominguez-Lerena *et al.* (2006) pointed out that the best single indicator of plant quality was the ratio of container depth to stem diameter, and the target plants had a container depth-to-stem diameter ratio of 4. This is consistent with our result above. Indeed, since the depth of the container cavities was 16 cm, the target collar diameter will be 4 mm superior to the critical minimum collar diameter of 3.24 mm. Thuja seedlings with a 16 cm stem height and 4 mm collar diameter, which result in a sturdiness index of 4 cm mm^{-1} inferior to the critical maximum sturdiness index of 5.97 cm mm^{-1} can be considered target plants. Nursery manager who are responsible for producing thuja seedlings for semiarid areas must strive to approach this morphological class. This is only achievable through the implementation of a highly precise fertilization, watering, and hardening plan in the nursery, which enables a balanced trade-off between shoot height and collar diameter. The production of thuja seedlings with such a target morphological class was possible by using the slow-release fertilizer NPK: 14-9-15 with 1.8 g per liter of growing media (Trubat *et al.*, 2011).

Shoot height relative growth is largely influenced by the initial morphological class in contrast to collar diameter relative growth which seems to be indifferent to the initial morphological traits of the seedlings (Table 5).

The sturdiness index variations of the seedlings (Table 5) seemed to be very reactive to their initial morphological class, showing an increasing tendency for small seedlings and a regressive behavior for large and extra-large seedlings. Under the dry conditions of the semiarid area, seedlings firstly invest in the mobilization of nutrients and water resources towards the root system that must develop rapidly to reach moister soil layers (Padilla & Pugnaire, 2007). This situation forces plants to reduce their aerial photosynthetic activity (Lamhamedi *et al.*, 1997) and invest in the development of the root system (Grossnickle, 2005). Extra-large seedlings may find it difficult to adapt quickly to this situation of water stress since their sturdiness index is largely high, preventing them from quickly recovering their imbalance (Rose *et al.*, 1993). As a re-sprouting coniferous, *T. articulata* has the ability to adapt to extreme drought conditions thanks to a lignotuber structure located just above the crown. This specialized structure which grows to a burl for adult trees (Charco, 1999; Paula *et al.*, 2016) is provided with dormant buds and callus, allowing plants to reject new stems and roots under stressful conditions. Seedlings with a large collar diameter are likely to have more stored water, carbohydrates and nutrients, allowing them to undertake a conservative strategy in the form of drought avoidance expressed as shoot growth reduction. According to Jaenicke (1999), a small sturdiness index indicates a sturdy plant with a higher than expected chance of survival, especially on windy or dry sites. A sturdiness index of > 6 indicates that the seedlings are weak and lanky, and therefore have less ability to withstand physical damage. In another research on black spruce, *Picea mariana* (Mill.), Roller (1977) found that seedlings with a sturdiness index of > 6 were seriously damaged when exposed to wind, drought, and frost. This corroborates with El Haddadi *et al.* (2022) who pointed out a strong negative correlation between the sturdiness index and root growth potential of thuja seedlings.

To enhance the planting success of seedlings in semiarid conditions, an effective approach involves modifying nursery cultivation techniques, such as increasing the root plug length by using containers with deeper cavities (Chirino *et al.*, 2008; Pinto *et al.*, 2011). The depth of the container determines the length of the tap root and thus the depth of the root system positioning in the soil (Peñuelas & Ocaña, 1996). The use of containers with a deeper cell cavity of 25 cm instead of 16 cm could enable thuja seedlings to benefit from an additional 9 cm of moister soil layers, allowing

their taproots to reach 66.74 cm in soil depth (equation 4), which corresponds to a gravimetric moisture content of 13.88% and an enhanced survival probability of 9.9% (odds ratio = 1.1).

Initial collar diameter was revealed as a good predictor of seedling rooting depth which, in turn, controls survival probability (Table 6). The ability of the initial diameter to predict rooting depth may be partly attributed to the strong linear relationship between initial diameter, root volume and first-order lateral roots (Jacobs *et al.*, 2005; Jacobs & Landis, 2009). El Haddadi *et al.* (2022) established that the collar diameter and root-to-shoot ratio (R/S) of thuja seedlings effectively predict root vitality and integrity, as measured by Root Electrolyte Leakage (REL), while the sturdiness index serves as a reliable indicator of root system development, as assessed by Root Growth Potential (RGP). In another study conducted on Aleppo pine (*Pinus halepensis* Mill.), a Mediterranean resinous species that coexists with thuja, Puértolas *et al.* (2003) recommend using seedlings with a collar diameter greater than 2.3 mm and a total dry weight exceeding 2.8 g for the restoration of abandoned lands.

Conclusion

The study results contribute to proposing practical guidelines for selecting thuja seedlings for restoration programs in semiarid areas. The plantation success rate could be improved by adapting the seedling size to the harsh pedo-climatic conditions of the planting site, with a tendency towards opting for sturdy short-stemmed seedlings (sturdiness index < 5.93 cm mm⁻¹) with a large collar diameter (> 3.24 mm). This study identified a critical minimum soil moisture level of 12%, which is found in soil horizons between 50 and 60 cm. This level guarantees the survival of the seedlings' root systems, enabling them to survive the dry season. The study of correlations between initial morphological parameters and taproot development highlighted collar diameter as a key predictor of deep root growth, explaining 65% of the observed variation.

Simple, rapid and non-destructive measurements of above-ground morphological parameters of thuja seedlings are still suited for assessing quality and predicting field performance. However, additional research should be conducted to highlight the benefits that could be provided by using thuja seedlings grown in deep-cavity containers in terms of seedling survival and outplanting performance in semiarid environments.

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